Appl. No. 09/151,612
Amdt. dated Tuesday, August 05, 2003
Reply to Telephonic Conference of August 1, 2003

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1. (currently amended) A method of increasing immune recognition of a mammalian cell in a subject comprising:

(a) obtaining a nonimmune cell from a subject in need of such treatment;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cell and thereby activating expression of a gene or gene product, wherein such activation is involved in antigen presentation~~, growth, and function of the cell,~~ and which increases the ability of [[a]] the cell to present antigen to an immune cell; and

(c) re-introducing the cell into the subject.

Claim 2. (previously amended) A method of increasing immune recognition of a nonimmune cell in a subject comprising:

(a) obtaining a cell from a subject in need of such treatment;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cell, thereby activating expression of a gene or gene product that increases immune recognition;

(c) introducing an antigen into the cell; and

(d) re-introducing the cell into the subject.

Claim 3. (cancelled)

Claim 4. (currently amended) A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell and thereby activating expression of a gene or gene product that increases immune recognition, wherein the polynucleotide does not contain a stimulatory CpG motif and wherein such activation is involved in antigen presentation~~, growth, and function of the cell~~; and

(c) re-introducing the immune cell into the subject.

Claim 5. (currently amended) A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell and thereby activating expression of a gene or gene product that increases immune recognition, wherein the polynucleotide is a noncoding polynucleotide sequence and wherein such activation is involved in antigen presentation~~, growth, and function of the cell~~; and

(c) re-introducing the immune cell into the subject.

Claim 6. (currently amended) The method of claim 1, 2, 4, or 5 wherein the gene or gene product associated with increased immune activation is selected from the group consisting of MHC class I, MHC class II, TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, RFXA, B7 co-stimulatory molecule, PKR, beta-interferon, MAP kinase, NF-kB, JAK, and STAT genes and gene products.

Claim 7. (currently amended) A method of increasing immune recognition of a monocyte or dendritic cell within a subject comprising:

(a) obtaining a monocyte or dendritic cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the monocyte or dendritic cell, wherein the

polynucleotide does not contain a CpG motif, and thereby activating expression of a gene, or gene product or gene and gene product that increases immune recognition, wherein such activation is involved in antigen presentation~~, growth, and function of the cell,~~ and which increases the ability of the monocyte or dendritic cell to present antigen to an immune cell of the subject; and

(c) re-introducing the cell into the subject.

Claim 8. (currently amended) A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell, thereby activating expression of a gene or gene product that increases immune recognition, wherein such activation is involved in antigen presentation~~, growth, and function of the cell,~~ and wherein the polynucleotide contains one or more CpG motifs, which if methylated do not decrease activity of the polynucleotide; and

(c) re-introducing the immune cell into the subject.

Claim 9. (currently amended) The method of claim 1, 2, 4, 5, 7, [[or]] 8, 46, 60, 76, 81 or 83, wherein the double-stranded polynucleotide is introduced by the method selected from the group consisting of transfection, microinjection, viral infection of the cell, and cell injury~~, phagocytosis of a bacterium, virus, or cell, and oncogene transformation~~.

Claim 10. (cancelled)

Claim 11. (currently amended) ~~The method of claim 1 wherein introduction of the double-stranded polynucleotide into the cell occurs by oncogene transformation~~ A method of increasing immune recognition of a nonimmune mammalian cell in a subject comprising:

(a) identifying a subject having a nonimmune mammalian cell in need of such treatment;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cell within the subject and thereby activating expression of a gene or gene product, which increases the ability of a cell to present antigen to an immune cell.

Claim 12. (currently amended) The method of claim 1, 4, 5, 6, 7, 8, 11, 46, [[or]] 60, 76, 81 or 83, wherein the cell expresses an autoantigen.

Claim 13. (currently amended) The method of claim 1, 2, or 11 wherein the cell is selected from the group consisting of somatic cell, antigen presenting cell and thyroid cell.

Claim 14. (previously amended) The method of claim 13 wherein the cell is a thyroid cell.

Claim 15. (currently amended) The method of ~~claim 7 or 8 wherein~~ claim 7, 8, or 11, wherein the gene or gene product that increases immune recognition is selected from the group consisting of MHC class I and class II genes and gene products, peptide processing genes and gene products, class II regulatory genes and gene products, ~~and~~ co-stimulatory molecule gene and gene products and beta-interferon.

Claim 16. (previously amended) The method of claim 6 or 15 wherein the gene or gene product is derived from the major histocompatibility complex (MHC) and wherein a MHC Class I expression increases greater than a MHC Class II expression as a function of time after introduction of concentration of the double-stranded polynucleotide.

Claim 17. (currently amended) The ~~method of claim 1~~ method of claim 6 or 15 wherein expression of the MHC molecule is accompanied by increased expression of an about 90 kilodalton tumor-associated immunostimulator.

Claim 18. (currently amended) The method of claim [[1,]] 4, 5, 7, or 8 wherein the method further comprises the step of introducing tumor cell RNA into the cell ex vivo.

Claim 19. (cancelled)

Claim 20. (cancelled)

Claim 21. (currently amended) The method of ~~claim 1, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 76, 81, or 83, wherein the cell can induce an autoimmune response when injected into the subject.

Claim 22. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 76, 81, or 83, wherein the cell recruits and activates T cells when injected into the subject.

Claim 23. (currently amended) The method of claim 1, 2, 4, 5, 7, 8, 11, 76, 81, or 83, wherein the cell produces at least one soluble mediator of immunity.

Claim 24. (currently amended) The method of claim 6 or 15 wherein increasing expression of the MHC molecule by double-stranded polynucleotide is additive to and independent of [[an]] a gamma- interferon-mediated increase in immune recognition.

Claim 25. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 76, 81, or 83, wherein the double-stranded polynucleotide is RNA that is introduced into the cell and wherein the polynucleotide initiates an antigen presenting response and does not initiate such antigen presenting response by acting through a cell surface receptor ~~does not induce a receptor-activated interferon response~~.

Claim 26. (cancelled)

Claim 27. (cancelled)

Claim 28. (cancelled)

Claim 29. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, 8, or 46 wherein~~ claim 1, 2, 4, 5, 7, 8, 46, 76, 81, or 83, wherein the method comprises the further step of treating the cells to prevent cell division prior to introducing the polynucleotide containing cell into a host organism.

Claim 30. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 46, 76, 81, or 83, wherein neither strand of the polynucleotide encodes a molecule involved in antigen presentation.

Claim 31. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 46, 76, 81, or 83, wherein the immune system of the subject recognizes one or more antigens presented by the cell.

Claim 32. (previously amended) The method of claim 75 wherein expression of both MHC Class I and Class II molecules in or on the cell are increased.

Claim 33. (currently amended) The method of ~~claim 26 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, 46, 76, 81, or 83, wherein the double-stranded polynucleotide comes from the mammalian cell's nucleus or mitochondria.

Claim 34. (previously amended) The method according to claim 74 and further comprising introducing an antigen into the mammalian cell prior to introduction of the activated APC into the subject.

Claim 35. (previously amended) The method of claim 34 wherein introduction causes an autoimmune reaction in the host animal.

Claim 36. (withdrawn) A screening method for a drug to regulate antigen presentation comprising:

a) introducing a double-stranded polynucleotide into a mammalian cell;

b) measuring expression in or on the mammalian cell of at least one molecule selected from the group consisting of major histocompatibility complex

(MHC) molecule and non-MHC molecule involved in antigen presentation;

c) mixing the mammalian cell with or without a candidate drug; and

d) measuring an increase or decrease in the mammalian cell's ability to present antigen after introduction of the double-stranded polynucleotide when incubations with or without the candidate drug are compared.

Claim 37. (withdrawn) The method of claim 36 wherein the introduction of a double-stranded polynucleotide is coincident with or after the incubation with or without a candidate drug.

Claim 38. (withdrawn) A method for drug screening comprising:

a) introducing double-stranded polynucleotide into a mammalian cell,

b) treating the cell with the drug before, coincident with or after introducing double-stranded polynucleotide, and

c) measuring expression of major histocompatibility complex (MHC) molecules and about a 90 kilodalton tumor-associated imunostimulator gene expression about 12 or more hours after treating the cell with the drug in step (b) is performed.

Claim 39. (withdrawn) The method of claim 38 wherein the drug is MMI, an MMI derivative, a thione or a thione derivative.

Claim 40. (withdrawn) A pharmaceutical composition wherein the composition includes a drug capable of preventing tissue damage caused by an autoimmune reaction, preventing atherosclerotic plague development, treating autoimmune disease, treating an infection, treating transplantation rejection, or treating tumor cells, comprising an effective amounts of Methimazole, methimazole derivatives, or tautomeric cyclic thiones.

Claim 41. (withdrawn) A DNA molecule comprising at least one of SEQ ID NOS: 1-16.

Claim 42. (currently amended) The method of ~~claim 1 wherein~~ claim 2, 4, 5, 7, 8, 11, 46, 76, 81, or 83, wherein the cell recruits and activates other T or B cells to enhance the immune response.

Claim 43. (previously amended) The method of claim 32 wherein increasing expression of the MHC molecule by double-stranded polynucleotide is additive to or independent of an interferon-mediated increase in expression of the MHC molecule.

Claim 44. (currently amended) The method of ~~claim 13 wherein~~ claim 25 wherein the double-stranded polynucleotide is RNA that increases ß-interferon production by the immune or antigen presenting cell.

Claim 45. (currently amended) The method of ~~claim 4, 5, or 13 wherein~~ claim 1, 4, 5, 8, 13, 46, 76, or 81, wherein the cell is a tumor cell and the subject has an increased ability to recognize and kill the tumor cell after such treatment.

Claim 46. (currently amended) A method of presenting antigen to the immune system of a mammal in need of immunotherapy comprising;

a) introducing double-stranded polynucleotide into a somatic mammalian cell ex vivo, which improves the ability of the mammalian cell to present antigen;

b) thereby increasing expression or activity of a molecule selected from the group consisting of MHC molecules, TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, RFXA, B7 co-stimulatory molecule, PKR, MAP kinase, NF-kB, JAK, beta-interferon, and STAT; and

c. introducing the somatic cells into the mammal; wherein the cells induce an immune response by the mammal to an antigen.

Claim 47 (withdrawn) A method of identifying differential expression of a sequence expressed in response to a double-stranded polynucleotide comprising:

(a) introducing the double-stranded polynucleotide into a mammalian cell;

(b) isolating expressed RNA sequences from the cell treated with the double-stranded polynucleotide and from a cell not treated with the double-stranded polynucleotide; and

(c) comparing the isolated RNA sequences of the treated cell with the untreated cell and identifying the sequences differentially expressed in the treated cell as compared to the untreated cell.

Claim 48 (withdrawn) The method of claim 47 wherein the sequence is expressed at a higher level in the double-stranded nucleotide-treated cell than in the untreated cell.

Claim 49 (withdrawn) The method of claim 47 wherein the sequence is expressed at a lower level in the double-stranded nucleotide-treated cell than in the treated cell.

Claim 50 (withdrawn) The method of claim 47 wherein the mammalian cell is selected from the group consisting of non-immune cell, immune cell, antigen presenting cell, and thyroid cell.

Claim 51 (withdrawn) The method of claim 47 wherein the double-stranded polynucleotide is introduced by a method selected from the group comprising transfection, microinjection, direct injection, viral infection, phagocytosis, oncogene transformation or cytoplasmic leakage.

Claim 52 (withdrawn) The method of claim 47 wherein control cells or cellscells treated with double-stranded polynucleotide are also treated with a drug to prevent changes induced by the double-stranded polynucleotide.

Claim 53 (withdrawn) The method of claim 52 wherein the drug is selected from the group consisting of MMI or an MMI derivative.

Claim 54 (withdrawn) The method of claim 53 wherein the drug is a tautomeric cyclic thione.

Claim 55 (withdrawn) A method of screening for a compound that regulates the effect of double-stranded polynucleotides, comprising:

(a) introducing the double-stranded polynucleotide into a mammalian cell;

(b) exposing or not exposing the cell to the compound before or with or after introducing the double-stranded polynucleotide;

(c) isolating the RNA of the cell

(d) quantitatively comparing the relative level of expression of double-stranded polynucleotide responsive genes expressed in the cell in the presence of absence of the compound; and

(e) identifying and selecting compounds shown to regulate the effect of double-stranded polynucleotides.

Claim 56 (withdrawn) The method of claim 55 wherein the double-stranded polynucleotide responsive genes are selected from the group comprising MHC genes, non-MHC genes, and growth-related genes.

Claim 57 (withdrawn) A method of screening for a compound that regulates the effect of double-stranded polynucleotides, comprising

(a) transfecting a non-professional immune cell with an antigen before or after introducing into the cell a double-stranded polynucleotide;

(b) immunizing an animal with the cell to induce an autoimmune disease;

(c) treating the animal with a compound; and

(d) determining whether the compound regulates the effect of the double-stranded polynucleotide.

Claim 58 (withdrawn) The method of claim 57 wherein the method of determining whether the compound regulates the effect of the double-stranded polynucleotide comprises:

(a) exposing or not exposing an animal to the compound;

(b) isolating the RNA of the cell from the relevant tissues;

(c) quantitatively comparing the relative level of expression of double-stranded polynucleotide responsive genes expressed in cell in the presence or absence of the compound; and

(d) identifying and selecting compounds shown to regulate the effect of double-stranded polynucleotides.

Claim 59 (withdrawn) The method of claim 57 wherein the method of determining whether the compound regulates the effect of the double-stranded polynucleotide comprises:

(a) exposing or not exposing the animal to the compound; and

(b) identifying and selecting compounds shown to prevent or alleviate the symptoms of the disease.

Claim 60. (previously amended) A method for treating a mammalian disease which is sensitive to immunotherapy which comprises:

a) removing diseased cells from a mammal identified as having a disease which is sensitive to immunotherapy;

b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cells;

c) treating the cells to prevent cell division but permit other metabolic activity; and

d) re-introducing the treated cells into the mammal; wherein the cells induce an immune response by the mammal to a self antigen.

Claim 61. (cancelled)

Claim 62. (currently amended) The method of ~~claim 60 wherein~~ claim 1, 2, 4, 5, 11, 46, 76, 81, or 83, wherein the method of treatment is used to enhance another treatment method which further enhances an immune response or an antigen presentation.

Claim 63-66. (cancelled)

Claim 67 (withdrawn) A method to assess viral replication which comprises:

(a) measuring the level of expression of a gene, the expression of which is affected by transfection with double-stranded polynucleotides, and

(b) comparing the level of expression with cells which are known to not have been infected with the virus.

Claim 68 (withdrawn) The method of claim 67 wherein the virus is single-stranded RNA virus.

Claim 69 (withdrawn) The method of Claim 68 wherein the virus is Hepatitis C or Hepatitis A.

Claim 70 (withdrawn) The method of claim 67 wherein the genes are selected from the group comprising MHC class I, MHC class II, TAP-1, TAP-2, HLA-DM, Ii, CIITA, RFX5, MAPK, NF-B, -IFN, JAK, STAT family of kinases.

Claim 71 (withdrawn) The method of claim 67 wherein the cells are transfected by single-stranded RNA from a virus.

Claim 72 (withdrawn) The method of claim 67 wherein a drug is added to prevent gene expression induced by a virus during the preparation procedure.

Claim 73 (withdrawn) A method of claim 72 wherein the drug is selected from a group consisting of MMI or MMI derivative or a tautomeric cyclic thione.

Claim 74. (previously amended) The method of claim 1, 2 or 7 additionally comprising forming an activated antigen presenting cell (APC).

Claim 75. (currently amended) The method of ~~claims 26 or 74 wherein the cell is a tumor cell and wherein~~ claim 45 or 74 wherein the treatment is in addition to treatment with CpG motifs.

Claim 76. (previously amended) A vaccine for treating cancer comprising:

(a) a somatic mammalian cell with the enhanced ability to present antigen to the immune system wherein a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length is introduced into the somatic mammalian cell *ex vivo*, which causes the cell to have an increased ability to present antigen; and

(b) a pharmaceutically acceptable carrier.

Claim 77. (withdrawn) A vaccine for treating cancer which is sensitive to immunotherapy which comprises;

a) an adjuvant comprising a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length;

b) an antigen of interest; and

c) a pharmaceutically acceptable carrier.

Claim 78. (withdrawn) A method for augmenting a vaccine response comprising administering an antigen and an adjuvant to a mammal in need of such treatment, wherein the adjuvant comprises a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length.

Claim 79. (cancelled)

Claim 80. (withdrawn) The method of claim 78 wherein the treatment is in addition to treatment with CpG motifs used to enhance immune cell responsiveness.

Claim 81. (currently amended) A method for treating cancer which is sensitive to immunotherapy which comprises:

a) obtaining a somatic cell from a subject in need of treatment;

b) introducing a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length into the somatic mammalian cell *ex vivo*, which causes the cell to have an increased ability to present antigen;

c) increasing the expression of one or more molecules involved in antigen presentation selected from the group consisting of MHC molecules, TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, RFX5, B7 costimulatory molecule, PKR, MAP Kinase, NF-κB, JAK, beta-interferon, and a STAT;

d) preparing the mammalian cell to make suitable for immunization; and

e) introducing the cell into a subject in need of such treatment.

Claim 82. (previously amended) The method of Claim 81 wherein the polynucleotide is single stranded RNA molecule that, when introduced into the cell, replicates to form a double stranded polynucleotide within the cell.

Claim 83. (previously amended) A method for treating a patient with a cancer which is sensitive to immunotherapy comprising:

a) removing monocytes from the patient;

b) introducing a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length into the monocytes *ex vivo*, which causes the monocytes to have an increased ability to present antigen;

c). introducing a tumor cell antigen into the monocytes wherein the antigen is selected from the group consisting of a protein, a peptide, an mRNA encoding antigen and a DNA encoding antigen; and

d). re-introducing the monocytes into the patient.

Claim 84. (currently amended) The method of ~~claim 1, 2, 4, 5, 7, or 8 wherein~~ claim 1, 2, 4, 5, 7, 11, 46, 76, 81, or 83, wherein the polynucleotide introduced into the cells is single stranded RNA molecule that, when introduced into the cell, replicates to form a double stranded polynucleotide within the cell.

Claim 85. (currently amended) The method of ~~claim 7 wherein~~ claim 76 wherein methylation of any CpG motifs within the polynucleotide does not effect the activity of the polynucleotide.

Claim 86. (previously added) The method of claim 7 wherein the double-stranded polynucleotide does not contain any stimulatory CpG motifs.

Claim 87. (currently amended) The ~~method~~ vaccine of claim 76, wherein the cell is a tumor cell.

Claim 88. (currently amended) The ~~method~~ vaccine of claim 76, wherein the cell is a fibroblast and wherein ~~the method further comprises the step of introducing~~ tumor cell RNA is introduced into the cell *ex vivo*.

Claim 89. (currently amended) The ~~method of claim 76 or 77~~ vaccine of claim 76, wherein the vaccine is ~~injected~~ adapted for injection in muscle tissue ~~of the mammal~~ of a mammal.

Claim 90. (currently amended) The method of ~~claim 1, 2, 7, or 78 wherein~~ claim 1, 2, 7, or 11, wherein methylation of any CpG motifs within the polynucleotide does not effect the activity of the polynucleotide.

Claim 91. (currently amended) The method of ~~1, 2, 7, or 78 wherein~~ claim 1, 2, 4, 5, 7, 8, 11, wherein the polynucleotide is a noncoding sequence.